=> d his

(FILE 'HOME' ENTERED AT 10:20:04 ON 17 DEC 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DDFB, DDFU, DGENE, DRUGB, DRUGMONOG2, ...' ENTERED AT 10:20:17 ON 17 DEC 2003

SEA (SIALIC ACID OR NANA OR NEU5AC)

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10	FILE ADISINSIGHT
2	FILE ADISNEWS
839	FILE AGRICOLA
193	FILE ANABSTR
615	FILE AQUASCI
218	FILE BIOBUSINESS
11	FILE BIOCOMMERCE
16851	FILE BIOSIS
465	FILE BIOTECHABS
465	FILE BIOTECHDS
4156	FILE BIOTECHNO
4497	FILE CABA
3319	FILE CANCERLIT
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262	FILE CONFSCI
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512	FILE DDFU
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565	FILE DRUGB
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593	FILE IFIPAT
5	FILE IMSPRODUCT
1258	FILE JICST-EPLUS
12	FILE KOSMET
3225	FILE LIFESCI
5	FILE MEDICONF
15267	FILE MEDLINE
102	FILE NIOSHTIC
121	FILE NTIS
144	FILE OCEAN
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12	FILE PHAR
1	FILE PHARMAML

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              24   FILE PHIN
            1138   FILE PROMT
               1   FILE RDISCLOSURE
            9259   FILE SCISEARCH
               2   FILE SYNTHLINE
            4472   FILE TOXCENTER
            3956   FILE USPATFULL
             113   FILE USPAT2
              93   FILE VETB
             117   FILE VETU
             918   FILE WPIDS
             918   FILE WPINDEX
L1              QUE (SIALIC ACID OR NANA OR NEU5AC)
                ---------

     FILE 'CAPLUS, BIOSIS, MEDLINE, EMBASE, SCISEARCH, CABA, TOXCENTER,
     BIOTECHNO, PASCAL, USPATFULL, CANCERLIT, LIFESCI, ESBIOBASE' ENTERED AT
     10:23:39 ON 17 DEC 2003
L2         18852 S L1 AND (SYNTHE? OR BIOSYNTHE?)
L3           142 S L2 AND (SYNTHASE AND PHOSPHATE SYNTHASE)
L4           103 DUP REM L3 (39 DUPLICATES REMOVED)
L5             2 S L2 AND (CMP-SA SYNTHASE)
L6             8 S L2 AND (SIALIC ACID PHOSPHATE SYNTHASE)
L7             2 DUP REM L5 (0 DUPLICATES REMOVED)
L8             2 DUP REM L7 (0 DUPLICATES REMOVED)
L9             2 DUP REM L6 (6 DUPLICATES REMOVED)

     FILE 'REGISTRY' ENTERED AT 10:31:10 ON 17 DEC 2003
                E CMP-SA SYNTHASE/CN
L10             1 S E4
                E SIALIC ACID PHOSPHATE SYNTHASE/CN
L11             1 S E4

     FILE 'CA, CAPLUS' ENTERED AT 10:34:16 ON 17 DEC 2003

     FILE 'REGISTRY' ENTERED AT 10:34:50 ON 17 DEC 2003
                SET SMARTSELECT ON
L12           SEL L11 1- CHEM :       7 TERMS
                SET SMARTSELECT OFF

     FILE 'CA, CAPLUS' ENTERED AT 10:34:51 ON 17 DEC 2003
L13             2 S L12
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=> file reg
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    48.72      52.23

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -1.30      -1.30

FILE 'REGISTRY' ENTERED AT 10:31:10 ON 17 DEC 2003
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PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
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STRUCTURE FILE UPDATES:   16 DEC 2003  HIGHEST RN 627482-61-5
DICTIONARY FILE UPDATES:  16 DEC 2003  HIGHEST RN 627482-61-5

TSCA INFORMATION NOW CURRENT THROUGH JULY 14, 2003

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
http://www.cas.org/ONLINE/DBSS/registryss.html

=> e CMP-SA synthase/CN
E1            1     CMP-NEUNAC SYNTHETASE (NEISSERIA MENINGITIDIS GENE NEUA)/CN
E2            1     CMP-PNP/CN
E3            0 --> CMP-SA SYNTHASE/CN
E4            1     CMP-SIALATE SYNTHASE/CN
E5            1     CMP-SIALATE SYNTHETASE/CN
E6            1     CMP-SIALIC ACID HYDROLASE/CN
E7            1     CMP-SIALIC ACID SYNTHETASE/CN
E8            1     CMP-SIALIC ACID SYNTHETASE ((ONCORHYNCHUS MYKISS)/CN
E9            1     CMP-SIALIC ACID SYNTHETASE (CAMPYLOBACTER JEJUNI STRAIN OH43
                    84)/CN
E10           1     CMP-SIALIC ACID SYNTHETASE (NEISSERIA MENINGITIDIS STRAIN 40
                    6Y CLONE PNSY-02)/CN
E11           1     CMP-SIALIC ACID SYNTHETASE NEUA (STREPTOCOCCUS AGALACTIAE GE
                    NE NEUA)/CN
E12           1     CMP-SIALIC ACID TRANSPORTER (CRICETULUS GRISEUS CELL LINE CH
                    O-K1)/CN

=> s E4;D
L10           1 "CMP-SIALATE SYNTHASE"/CN


L10  ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9067-82-7  REGISTRY
CN   Cytidylyltransferase, acylneuraminate (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Acylneuraminate cytidylyltransferase
CN   CMP sialate pyrophosphorylase
CN   CMP-acylneuraminate synthetase
CN   CMP-N-acetylneuraminate synthetase
```

CN CMP-N-acetylneuraminate synthetase
CN CMP-N-acetylneuraminic acid synthase
CN CMP-N-acetylneuraminic acid synthetase
CN CMP-NANA synthetase
CN CMP-Neu5A synthetase
CN **CMP-sialate synthase**
CN CMP-sialate synthetase
CN CMP-sialic acid synthetase
CN Cytidine 5'-monophospho-N-acetylneuraminic acid synthetase
CN Cytidine 5'-monophospho-N-acetylneuraminic acid synthetase
CN Cytidine 5'-monophosphosialic acid synthetase
CN Cytidine 5-monophosphate N-acetylneuraminic acid synthetase
CN Cytidine monophosphate-N-acetylneuraminic acid synthetase
CN Cytidine monophospho-sialic acid synthetase
CN Cytidine monophosphoacetylneuraminic synthetase
CN Cytidine monophosphosialate pyrophosphorylase
CN Cytidine monophosphosialate synthase
CN Cytidine-5'-monophosphosialate synthase
CN Cytidylyltransferase, acylneuraminate
CN E.C. 2.7.7.43
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, EMBASE, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
215 REFERENCES IN FILE CA (1907 TO DATE)
6 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
215 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> e sialic acid phosphate synthase/CN
E1 1 SIALIC ACID LYASE/CN
E2 1 SIALIC ACID PERMEASE (ESCHERICHIA COLI CLONE PSX600 GENE NANT)/CN
E3 0 --> SIALIC ACID PHOSPHATE SYNTHASE/CN
E4 1 SIALIC ACID PHOSPHATE SYNTHASE (DROSOPHILA MELANOGASTER STRAIN OREGON-R)/CN
E5 1 SIALIC ACID SYNTHASE/CN
E6 1 SIALIC ACID SYNTHASE (CAMPYLOBACTER JEJUNI STRAIN MSC-57360 GENE NEUB1)/CN
E7 1 SIALIC ACID SYNTHASE (CAMPYLOBACTER JEJUNI STRAIN OH4384)/CN
E8 1 SIALIC ACID SYNTHASE (CHLOROBIUM TEPIDUM STRAIN TLS GENE CT0825)/CN
E9 1 SIALIC ACID SYNTHASE (CLOSTRIDIUM ACETOBUTYLICUM STRAIN ATCC 824 GENE CAC2187)/CN
E10 1 SIALIC ACID SYNTHASE (DROSOPHILA MELANOGASTER STRAIN OREGON-R)/CN
E11 1 SIALIC ACID SYNTHASE (HELICOBACTER HEPATICUS STRAIN ATCC51449 GENE HH0908)/CN
E12 1 SIALIC ACID SYNTHASE (HELICOBACTER PYLORI STRAIN J99 GENE NEUB)/CN

=> s E4;D
L11 1 "SIALIC ACID PHOSPHATE SYNTHASE (DROSOPHILA MELANOGASTER STRAIN OREGON-R)"/CN

L11 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS on STN
RN 433280-04-7 REGISTRY
CN Synthase, N-acylneuraminate 9-phosphate (Drosophila melanogaster strain Oregon-R) (9CI) (CA INDEX NAME)
OTHER NAMES:

CN GenBank AAK92125
CN GenBank AAK92125 (Translated from: GenBank AF397531)
CN Neu5Ac synthase (Drosophila melanogaster strain Oregon-R)
CN **sialic acid phosphate synthase (Drosophila melanogaster strain Oregon-R)**
CN sialic acid synthase (Drosophila melanogaster strain Oregon-R)
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> file ca caplus

☐1: AAK92125. Neu5Ac synthase [...[gi:15213695] BLink, Domains, Links

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LOCUS       AAK92125                372 aa            linear   INV 20-MAR-2002
DEFINITION  Neu5Ac synthase [Drosophila melanogaster].
ACCESSION   AAK92125
VERSION     AAK92125.1  GI:15213695
DBSOURCE    accession AF397531.1
KEYWORDS    .
SOURCE      Drosophila melanogaster (fruit fly)
  ORGANISM  Drosophila melanogaster
            Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota;
            Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha;
            Ephydroidea; Drosophilidae; Drosophila.
REFERENCE   1  (residues 1 to 372)
  AUTHORS   Kim,K., Lawrence,S.M., Park,J., Pitts,L., Vann,W.F.,
            Betenbaugh,M.J. and Palter,K.B.
  TITLE     Expression of a functional Drosophila melanogaster
            N-acetylneuraminic acid (Neu5Ac) phosphate synthase gene: evidence
            for endogenous sialic acid biosynthetic ability in insects
  JOURNAL   Glycobiology 12 (2), 73-83 (2002)
  MEDLINE   21883822
   PUBMED   11886840
REFERENCE   2  (residues 1 to 372)
  AUTHORS   Kim,K., Lawrence,S.M., Park,J., Pitts,L., Vann,W.F.,
            Betenbaugh,M.J. and Palter,K.B.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUL-2001) Department of Biology, Temple University,
            1900 N. 12th St., Philadelphia, PA 19122, USA
COMMENT     Method: conceptual translation supplied by author.
FEATURES             Location/Qualifiers
     source          1..372
                     /organism="Drosophila melanogaster"
                     /strain="Oregon R"
                     /db_xref="taxon:7227"
                     /chromosome="3"
                     /map="3R; 87B15"
                     /dev_stage="0-16 hour embryo"
     Protein         1..372
                     /product="Neu5Ac synthase"
                     /name="SAS"
     CDS             1..372
                     /coded_by="AF397531.1:35..1153"
ORIGIN
        1 mllndiisgk lvdsvyiiae igqnhqgcve takkmiweak kagchcvkfq ksdlpakftr
       61 saldreyisd hawgktygeh keylefskdq ylqlqahcke lnvdftasam dersleflsa
      121 lnvpfikigs gdannfpllk kaanlnlplv istgmqtmqt verivqtmre sgkedyalmh
      181 cvssyptdpk dcslqlisvl rtrfpnvaig ysghelgvii sqaavllgar iverhftldk
      241 sqkgsdhrcs lepqelkalt taitnfklss vpmppqeivk klngdeelea alqhveskti
      301 lpcelpcrnk lgksivaarn lnkgyrlqla dmaikvseps gltaedfldl vgkeladnig
      361 edepilgnsi in
//
```

=> d l13 ibib ab 1-2

L13 ANSWER 1 OF 2 CA COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 137:17958 CA
TITLE: Expression of a functional Drosophila melanogaster N-acetylneuraminic acid (Neu5Ac) phosphate synthase gene: Evidence for endogenous sialic acid biosynthetic ability in insects
AUTHOR(S): Kim, Kildong; Lawrence, Shawn M.; Park, Jung; Pitts, Lee; Vann, Willie F.; Betenbaugh, Michael J.; Palter, Karen B.
CORPORATE SOURCE: Department of Biology, Temple University, Philadelphia, PA, 19122, USA
SOURCE: Glycobiology (2002), 12(2), 73-83
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, we report the first cloning and characterization of a N-acetylneuraminic acid phosphate synthase gene from Drosophila melanogaster, an insect in the protostome lineage. The gene is ubiquitously expressed at all stages of Drosophila development and in Schneider cells. Similar to the human homolog, the gene encodes an enzyme with dual substrate specificity that can use either N-acetylmannosamine 6-phosphate or mannose 6-phosphate to generate phosphorylated forms of both the sialic acids, N-acetylneuraminic acid and 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, resp., when expressed in either bacterial or baculoviral expression systems. The identification of a functional sialic acid synthase in Drosophila indicates that insects have the biosynthetic capability to produce sialic acids endogenously. Although sialylation is widely distributed in organisms of the deuterstome lineage, genetic evidence concerning the presence or absence of sialic acid metab. in organisms of the protostome lineage has been lacking. Homol. searches of the Drosophila genome identified putative orthologues of other genes required for sialylation of glycoconjugates.
REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:266549 CAPLUS
DOCUMENT NUMBER: 137:17958
TITLE: Expression of a functional Drosophila melanogaster N-acetylneuraminic acid (Neu5Ac) phosphate synthase gene: Evidence for endogenous sialic acid biosynthetic ability in insects
AUTHOR(S): Kim, Kildong; Lawrence, Shawn M.; Park, Jung; Pitts, Lee; Vann, Willie F.; Betenbaugh, Michael J.; Palter, Karen B.
CORPORATE SOURCE: Department of Biology, Temple University, Philadelphia, PA, 19122, USA
SOURCE: Glycobiology (2002), 12(2), 73-83
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, we report the first cloning and characterization of a N-acetylneuraminic acid phosphate synthase gene from Drosophila melanogaster, an insect in the protostome lineage. The gene is ubiquitously expressed at all stages of Drosophila development and in Schneider cells. Similar to the human homolog, the gene encodes an enzyme with dual substrate specificity that can use either N-acetylmannosamine 6-phosphate or mannose 6-phosphate to generate phosphorylated forms of both the sialic acids, N-acetylneuraminic acid and 2-keto-3-deoxy-D-

glycero-D-galacto-nononic acid, resp., when expressed in either bacterial or baculoviral expression systems. The identification of a functional sialic acid synthase in Drosophila indicates that insects have the biosynthetic capability to produce sialic acids endogenously. Although sialylation is widely distributed in organisms of the deuterstome lineage, genetic evidence concerning the presence or absence of sialic acid metab. in organisms of the protostome lineage has been lacking. Homol. searches of the Drosophila genome identified putative orthologues of other genes required for sialylation of glycoconjugates.

REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l8 ibib ab 1-2

L8 ANSWER 1 OF 2 USPATFULL on STN
ACCESSION NUMBER: 2002:258816 USPATFULL
TITLE: Engineering intracellular sialylation pathways
INVENTOR(S): Betenbaugh, Michael J., Baltimore, MD, UNITED STATES
Lawrence, Shawn, Dobbs Ferry, NY, UNITED STATES
Lee, Yuan C., Timonium, MD, UNITED STATES
Coleman, Timothy A., Gaithersburg, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002142386	A1	20021003
APPLICATION INFO.:	US 2001-930440	A1	20010816 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-227579P	20000825 (60)
	US 1999-169624P	19991208 (60)
	US 1999-122582P	19990302 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 47
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 40 Drawing Page(s)
LINE COUNT: 4472
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technology and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compositions comprise cells of interest producing sialylated glycoproteins. The methods and compositions are useful for heterologous expression of glycoproteins.

L8 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free

medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA **synthesis** have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) **synthesis** when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l9 ibib ab 1-2

L9 ANSWER 1 OF 2 USPATFULL on STN

ACCESSION NUMBER:	2002:258816 USPATFULL		
TITLE:	Engineering intracellular sialylation pathways		
INVENTOR(S):	Betenbaugh, Michael J., Baltimore, MD, UNITED STATES Lawrence, Shawn, Dobbs Ferry, NY, UNITED STATES Lee, Yuan C., Timonium, MD, UNITED STATES Coleman, Timothy A., Gaithersburg, MD, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002142386	A1	20021003
APPLICATION INFO.:	US 2001-930440	A1	20010816 (9)
	NUMBER		DATE
PRIORITY INFORMATION:	US 2000-227579P		20000825 (60)
	US 1999-169624P		19991208 (60)
	US 1999-122582P		19990302 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850		
NUMBER OF CLAIMS:	47		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	40 Drawing Page(s)		
LINE COUNT:	4472		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technology and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate

engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compositions comprise cells of interest producing sialylated glycoproteins. The methods and compositions are useful for heterologous expression of glycoproteins.

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA **synthesis** have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid** **phosphate synthase** with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) **synthesis** when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

=> d l15 ibib ab 1-20

L15 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:939557 CAPLUS
TITLE: Engineering **Sialic Acid** **Synthetic** Ability into **Insect** **Cells**: Identifying Metabolic Bottlenecks and Devising Strategies To Overcome Them
AUTHOR(S): Viswanathan, Karthik; Lawrence, Shawn; Hinderlich, Stephan; Yarema, Kevin J.; Lee, Yuan C.; Betenbaugh, Michael J.
CORPORATE SOURCE: Departments of Chemical and Biomolecular Engineering, Biomedical Engineering and Biology, Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Biochemistry (2003), 42(51), 15215-15225
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Previous studies have indicated negligible levels of both sialylation and the precursor N-acetylneuraminic acid (**Neu5Ac**) in a no. of **insect cell** lines grown in serum-free medium. The overexpression of the human **sialic acid** 9-phosphate synthase (SAS) in combination with N-acetylmannosamine (ManNAc) feeding has been shown to overcome this limitation. In this study we evaluated the potential bottlenecks in the **sialic acid** **synthesis** pathway in a Spodoptera frugiperda (Sf9) **insect** **cell** line and devised strategies to overcome them by overexpression of the enzymic pathway enzymes combined with appropriate substrate feeding. Coexpression of SAS and UDP-GlcNAc 2-epimerase/ManNAc kinase, the bifunctional enzyme initiating **sialic acid** **biosynthesis** in mammals, resulted in **Neu5Ac** **synthesis** without use of any external media supplementation to demonstrate that **Neu5Ac** could be generated intracellularly in Sf9 cells using natural metabolic precursors. N-Acetylglucosamine (GlcNAc) feeding in combination with this coexpression resulted in much higher levels of **Neu5Ac** compared to levels obtained with ManNAc feeding with SAS expression alone. The lower **Neu5Ac** levels obtained with ManNAc feeding suggested limitations in the transport and phosphorylation of ManNAc. The bottleneck in phosphorylation was likely due to utilization of GlcNAc kinase for phosphorylation of ManNAc in **insect cells** and was overcome by expression of ManNAc kinase. The transport limitation was addressed by the addn. of tetra-O-acetylated ManNAc, which is easily taken up by the cells. An alternative **sialic acid**, 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN), could also be generated in **insect** **cells**, suggesting the potential for controlling not only the prodn. of **sialic acids** but also the type of **sialic acid** generated. The levels of KDN could be increased with virtually no **Neu5Ac** generation when Sf9 cells were fed excess GlcNAc. The results of these studies may be used to enhance the sialylation of target glycoproteins in insect and other eukaryotic expression systems.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 139:257257 CA
TITLE: Recombinant (2.fwdarw.3)-.alpha.-sialyltransferase immobilized on nickel-Agarose for preparative **synthesis** of sialyl Lewisx and Lewisa precursor oligosaccharides
AUTHOR(S): Ivannikova, Tatiana; Bintein, Fabrice; Malleron, Annie; Juliant, Sylvie; Cerutti, Martine;

Harduin-Lepers, Anne; Delannoy, Philippe; Auge, Claudine; Lubineau, Andre

CORPORATE SOURCE: UMR 8614, Laboratoire de Chimie Organique Multifonctionnelle, Universite de Paris-Sud, Orsay, F-91405, Fr.

SOURCE: Carbohydrate Research (2003), 338(11), 1153-1161
CODEN: CRBRAT; ISSN: 0008-6215

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The specificity of recombinant (2.fwdarw.3)-.alpha.-sialyltransferase (ST3Gal-III), expressed in baculovirus-infected **insect cells**, has been detd. with various oligosaccharide acceptors and sugar-nucleotide donors using a fluorescence based assay. Recombinant ST3Gal-III tagged with a polyhistidine tail was immobilized on Ni2+-NTA-Agarose as an active enzyme for use in the **synthesis** of three sialylated oligosaccharides: (i) the divalent mol. [.alpha.-**Neu5Ac**-(2.fwdarw.3)-d-Galp-(1.fwdarw.4)-.beta.-d-GlcpNAc-O-CH2]2-C-(CH2OBn)2 (compd. 12); (ii) the dansylated deriv., .alpha.-**Neu5Ac**-(2.fwdarw.3)-d-Galp-(1.fwdarw.3)-.beta.-d-GlcpNAc-O-(CH2)6-NH-dansyl and; (iii) the tetrasacharide .alpha.-**Neu5Ac**-(2.fwdarw.3)-.beta.-d-Galp-(1.fwdarw.4)-.beta.-d-GlcpNAc-(1.fwdarw.2)-.alpha.-d-Manp-O-CH3. Compd. 12 was itself prepd. from the divalent N-acetyllactosamine mol. built on pentaerythritol by a chemo-enzymic route.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:495062 CAPLUS

TITLE: A transgenic **insect cell** line engineered to produce CMP-**sialic acid** and sialylated glycoproteins

AUTHOR(S): Aumiller, Jared J.; Hollister, Jason R.; Jarvis, Donald L.

CORPORATE SOURCE: Department of Molecular Biology, University of Wyoming, Laramie, WY, 82071-3944, USA

SOURCE: Glycobiology (2003), 13(6), 497-507
CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have previously engineered transgenic **insect cell** lines to express mammalian glycosyltransferases and showed that these cells can sialylate N-glycoproteins, despite the fact that they have little intracellular **sialic acid** and no detectable CMP-**sialic acid**. In the accompanying study, we presented evidence that these cell lines can salvage **sialic acids** for de novo glycoprotein sialylation from extracellular sialoglycoproteins, such as fetuin, found in fetal bovine serum. This finding led us to create a new transgenic **insect cell** line designed to **synthesize** its own **sialic acid** and CMP-**sialic acid**. SfSWT-1 cells, which encode five mammalian glycosyl-transferases, were transformed with two addnl. mammalian genes that encode **sialic acid** synthase and CMP-**sialic acid synthetase**. The resulting cell line expressed all seven mammalian genes, produced CMP-**sialic acid**, and sialylated a recombinant glycoprotein when cultured in a serum-free growth medium supplemented with N-acetylmannosamine. Thus the addn. of mammalian genes encoding two enzymes involved in CMP-**sialic acid** **biosynthesis** yielded a new transgenic **insect cell** line, SfSWT-3, that can sialylate recombinant glycoproteins in the absence of fetal bovine serum. This new cell line will be widely useful as an improved host for baculovirus-mediated recombinant

glycoprotein prodn.
REFERENCE COUNT: 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 4 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 139:146659 CA
TITLE: Evidence for a **sialic acid** salvaging pathway in lepidopteran **insect cells**
AUTHOR(S): Hollister, Jason; Conradt, Harald; Jarvis, Donald L.
CORPORATE SOURCE: Department of Molecular Biology, University of Wyoming, Laramie, WY, 82071, USA
SOURCE: Glycobiology (2003), 13(6), 487-495
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors previously described a transgenic **insect cell** line, Sf.beta.4GalT/ST6, that expresses mammalian .beta.-1,4-galactosyltransferase and .alpha.2,6-sialyltransferase genes and produces glycoproteins with terminally sialylated N-glycans. The ability of these cells to produce sialylated N-glycans was surprising because **insect cells** contain only small amts. of **sialic acid** and no detectable CMP-**sialic acid**. Thus, it was of interest to investigate potential sources of **sialic acids** for sialoglycoprotein **synthesis** by these cells. The authors found that Sf.beta.4GalT/ST6 cells can produce sialylated N-glycans when cultured in the presence but not in the absence of fetal bovine serum. The serum component(s) supporting N-glycan sialylation by Sf.beta.4GalT/ST6 cells is relatively large-it was not removed by dialysis in a 50,000-mol.-wt. cutoff membrane. Serum-free media supplemented with purified fetuin but not asialofetuin supported N-glycan sialylation by Sf.beta.4GalT/ST6 cells. The terminally sialylated N-glycans isolated from fetuin also supported glycoprotein sialylation by Sf.beta.4GalT/ST6 cells. Finally, serum-free medium supplemented with N-acetylneuraminic acid or N-acetylmannosamine supported glycoprotein sialylation by Sf.beta.4GalT/ST6 cells but to a much lower degree than serum or fetuin. These results provide the first evidence of a **sialic acid** salvaging pathway in **insect cells**, which begins to explain how Sf.beta.4GalT/ST6 and other transgenic **insect cell** lines can sialylate recombinant glycoproteins in the absence of a more obvious source of CMP-**sialic acid**.
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 5 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 137:321839 CA
TITLE: Effect of microgravity environment on sialylation of N-linked glycoproteins in the baculovirus expression vector system
INVENTOR(S): Wood, H. Alan
PATENT ASSIGNEE(S): Boyce Thompson Institute for Plant Research, Inc., USA
SOURCE: U.S., 20 pp., Cont.-in-part of U.S. 6,261,805.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6472175	B1	20021029	US 2000-714085	20001117

US 6261805 B1 20010717 US 1999-353897 19990715
PRIORITY APPLN. INFO.: US 1999-353897 A2 19990715
AB The present disclosure the use of a microgravity environment or dexamethasone or N-acetylmannosamine supplemented **insect cell** culture media to optimize the processing of the oligosaccharide moieties attached to glycoproteins in the baculovirus expression vector system (BEVS). Specifically, the present invention analyzes the effects of supplementing **insect cell** culture media with dexamethasone or N-acetylmannosarnine on complex glycosylation of proteins prepd. via BEVS, including the addn. of terminal **sialic acid** residues to N-linked oligosaccharides. The invention increases the fundamental understanding of the effects of a microgravity environment on the biochem. and cellular factors and processes involved with eukaryotic protein **synthesis**, secretion and co- and post-translational processing.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:614154 CAPLUS
TITLE: Engineering the sialylation pathway in **insect cells**
AUTHOR(S): Viswanathan, Karthik; Lawrence, Shawn; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18-22, 2002 (2002), BIOT-197. American Chemical Society: Washington, D. C.
CODEN: 69CZPZ
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Sialylation has been shown in cells to be directly related to the levels of sialylation substrate, namely N-acetylneuraminic acid (**Neu5Ac**). Previous studies have indicated negligible intracellular levels of both **sialic acids** including **Neu5Ac** and CMP-**sialic acids** in a no. of **insect cell** lines grown in serum-free medium. In this study we identified the bottlenecks in the **sialic acid synthesis** pathway and were able to overcome this by overexpression of the genes of the pathway enzymes combined with appropriate substrate feeding. An alternative **sialic acid** 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) can also be generated in **insect cells**. By suggesting the potential for controlling not only the prodn. of **sialic acids** but also the type of **sialic acid** that is generated. The results of these studies can be used to optimize the sialylation process in insect as well as mammalian cell culture systems.

L15 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:614089 CAPLUS
TITLE: Strategies for humanization of **insect-cell** produced glycoproteins
AUTHOR(S): Tomiya, Noboru; Rahman, Badrul; Viswanathan, Karthik; Ahmad, Naz; Lee, Yuan C.; Betenbaugh, Michael J.
CORPORATE SOURCE: Biology, Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18-22, 2002 (2002), BIOT-132. American Chemical Society: Washington, D. C.
CODEN: 69CZPZ
DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB N-Glycosylation is one of major post translational modification of proteins, and sialylated complex-type N-glycans are often found in glycoproteins expressed by mammalian cells. **Insect cell** -baculovirus expression vector system is widely used for expression of foreign proteins because of its high yield. However, **insect cells** do not **synthesize** such sialylated N-glycans. Instead, glycoproteins expressed by **insect cells** have truncated N-glycans and high-mannose type N-glycans. These differences of N-glycan structures are caused by (i) insufficient glycosyltransferase activities, (ii) lacks of several enzymes to generate CMP-**sialic acid**, (iii) presence of high level of glycosidases in **insect cell**-BEV system. Overexpression of glycosyltransferases and enzymes required for CMP-**sialic acid synthesis**, and suppression of glycosidase activity in this protein expression system would provide a new **insect cell**-BEV system that expresses foreign proteins having complex mammalian-type N-glycans.

L15 ANSWER 8 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 135:41800 CA
TITLE: Recombinant cells with altered intracellular sialylation pathways and their use in producing glycoproteins
INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Coleman, Timothy A.; Palter, Karen; Jarvis, Don
PATENT ASSIGNEE(S): Human Genome Sciences, Inc, USA; Johns Hopkins University; Temple University; University of Wyoming
SOURCE: PCT Int. Appl., 182 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001042492	A1	20010614	WO 2000-US33136	20001207

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-169839P P 19991209

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technol. and substrate feeding approaches to enable the prodn. of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examn. of a selection of **insect cell** lines and the use of bioinformatics to identify gene sequences for crit. processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous expression of glycoproteins. Thus, the cDNA for a human **sialic acid** 9-phosphate **synthetase** which produces phosphorylated KDN and **Neu5Ac** from ManNAc-6-P and Man-6-P was cloned and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 9 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 134:111247 CA
TITLE: Sialylation of N-linked glycoproteins in the baculovirus expression vector system
INVENTOR(S): Wood, H. Alan
PATENT ASSIGNEE(S): Boyce Thompson Institute for Plant Research, Inc., USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001005956	A2	20010125	WO 2000-US19109	20000713
WO 2001005956	A3	20010907		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6261805	B1	20010717	US 1999-353897	19990715

PRIORITY APPLN. INFO.: US 1999-353897 A 19990715

AB The present disclosure utilizes a novel approach to protein prepn. in the baculovirus expression vector system (BEVS). Specifically, the present invention analyzes the effects of supplementing **insect cell** culture media with dexamethasone or N-acetylmannosamine on complex glycosylation of proteins prepd. via BEVS, including the addn. of terminal **sialic acid** residues to N-linked oligosaccharides. Culturing under microgravity conditions significantly also alters the processing of oligosaccharides during the **synthesis** of glycoproteins. A model system was developed comprising a recombinant isolate of the Autographa californica nuclear polyhedrosis virus expressing a secreted form of human placental alk. phosphatase (SEAP) in BIT-TN-5B1-4 cell lines from Tricoplusia ni. When SEAP was produced in the Tn-4h cells in HARV (high aspect ratio vessels) bioreactors, glycan fractions were detected with terminal .alpha.2,3- and 2,6-linked **sialic acid** residues, accounting for 20% of the total glycans attached to the single glycosylation site on SEAP. These levels are comparable to the 18% sialoglycan structures isolated from SEAP produced in the human placenta.

L15 ANSWER 10 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 136:213820 CA
TITLE: The occurrence and significance of **sialic acids** in insects
AUTHOR(S): Schauer, Roland
CORPORATE SOURCE: Biochemisches Inst., Christian-Albrechts-Univ., Kiel, D-24098, Germany
SOURCE: Trends in Glycoscience and Glycotechnology (2001), 13(73), 507-517
CODEN: TGGLEE; ISSN: 0915-7352
PUBLISHER: FCCA
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English/Japanese

AB A review. **Sialic acids**, mainly N-acetylneuraminic

acid, have been found during the development of a few insect species. In Drosophila melanogaster and the cicada Philaenus spumarius we detected polysialic acids, using lectins, antibodies, and various methods for the isolation and anal. of the monosaccharide subunits, in areas of neuronal development and in Malpighian tubules, resp. However, **sialic acids** were not found in the adult animals of these species. Although the expression of these acidic sugars has unequivocally been demonstrated in vivo, their occurrence in **insect cell** cultures was, in most cases, not clearly demonstrated. The **biosynthesis** of **sialic acids** in cell cultures would be of great biotechnol. significance, since the prodn. of recombinant glycoproteins with mammalian type, complex, sialylated N-glycans using the baculovirus expression system would be of great benefit. From the studies dedicated to this problem it appears that the engineering of such glycans may only be possible by the expression of exogenous genes in **insect cells**. Several genes encoding **sialic acid**-metabolizing enzymes have therefore been transfected. The work on **insect cells** shows that **sialic acids** are not only restricted to the Deuterostomia branch of the animal kingdom but also occur in some Protostomia species, as in insects. This throws new light on the evolution of this acidic sugar.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 11 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 7

ACCESSION NUMBER: 136:364440 CA

TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in **insect cells**

AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.

CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA

SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080

PUBLISHER: Kluwer Academic Publishers

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of **insect cells** grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA **synthesis** have been engineered into **insect cells** using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in **insect cells** using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when

N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in **insect cells**. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) **synthesis** when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 12 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 134:294560 CA

TITLE: Glycoproteins from **insect cells**: sialylated or not?

AUTHOR(S): Marchal, Ingrid; Jarvis, Donald L.; Cacan, Rene; Verbert, Andre

CORPORATE SOURCE: Laboratoire de Glycobiologie Structurale et Fonctionnelle, CNRS UMR no8576, Universite des Sciences et Technologies de Lille, Villeneuve d'Ascq, F-59655, Fr.

SOURCE: Biological Chemistry (2001), 382(2), 151-159
CODEN: BICHF3; ISSN: 1431-6730

PUBLISHER: Walter de Gruyter GmbH & Co. KG

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 67 refs. Our growing comprehension of the biol. roles of glycan moieties has created a clear need for expression systems that can produce mammalian-type glycoproteins. In turn, this has intensified interest in understanding the protein glycosylation pathways of the heterologous hosts that are commonly used for recombinant glycoprotein expression. Among these, **insect cells** are the most widely used and, particularly in their role as hosts for baculovirus expression vectors, provide a powerful tool for biotechnol. Various studies of the glycosylation patterns of endogenous and recombinant glycoproteins produced by **insect cells** have revealed a large variety of O- and N-linked glycan structures and have established that the major processed O- and N-glycan species found on these glycoproteins are (Gal.beta.1,3)GalNAc-O-Ser/Thr and Man3(Fuc)GlcNAc2-N-Asn, resp. However, the ability or inability of **insect cells** to **synthesize** and compartmentalize **sialic acids** and to produce sialylated glycans remains controversial. This is an important issue because terminal **sialic acid** residues play diverse biol. roles in many glycoconjugates. While most work indicates that **insect cell**-derived glycoproteins are not sialylated, some well-controlled studies suggest that sialylation can occur. In evaluating this work, it is important to recognize that oligosaccharide structural detn. is tedious work, due to the infinite diversity of this class of compds. Furthermore, there is no universal method of glycan anal.; rather, various strategies and techniques can be used, which provide glycobiologists with relatively more or less precise and reliable results. Therefore, it is important to consider the methodol. used to assess glycan structures when evaluating these studies. The purpose of this review is to survey the studies that have contributed to our current view of glycoprotein sialylation in **insect cell** systems, according to the methods used. Possible reasons for the disagreement on this topic in the literature, which include the diverse origins of biol. material and exptl. artifacts, will be discussed. In the final anal., it appears that if **insect cells** have the genetic potential to perform sialylation of glycoproteins, this is a highly specialized function that probably occurs rarely. Thus, the prodn. of sialylated recombinant glycoproteins in the baculovirus-

insect cell system will require metabolic engineering efforts to extend the native protein glycosylation pathways of **insect cells**.

REFERENCE COUNT: 67 THERE ARE 67 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 13 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 135:16448 CA
TITLE: **Sialic acid**-dependent binding of baculovirus-expressed recombinant antigens from Plasmodium falciparum EBA-175 to Glycophorin A
AUTHOR(S): Ockenhouse, C. F.; Barbosa, A.; Blackall, D. P.; Murphy, C. I.; Kashala, O.; Dutta, S.; Lanar, D. E.; Daugherty, J. R.
CORPORATE SOURCE: Department of Immunology, Walter Reed Army Institute of Research, Silver Spring, MD, USA
SOURCE: Molecular and Biochemical Parasitology (2001), 113(1), 9-21
CODEN: MBIPDP; ISSN: 0166-6851
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Plasmodium falciparum Erythrocyte Binding Antigen-175, EBA-175, is a sol. merozoite stage parasite protein which binds to glycophorin A surface receptors on human erythrocytes. We have expressed two conserved cysteine-rich regions, region II and region VI, of this protein as sol. His-tagged polypeptides in **insect cell** culture, and have tested their function in erythrocyte and glycophorin A binding assays. Recombinant region II polypeptides comprised of the F2 sub-domain or the entire region II (F1 and F2 sub-domains together) bound to erythrocytes and to purified glycophorin A in a manner similar to the binding of native P. falciparum EBA-175 to human red cells. Removal of **sialic acid** residues from the red cell surface totally abolished recombinant region II binding, while trypsin treatment of the erythrocyte surface reduced but did not eliminate recombinant region II binding. **Synthetic** peptides from three discontinuous regions of the F2 sub-domain of region II inhibited human erythrocyte cell binding and glycophorin A receptor recognition. Immune sera raised against EBA-175 recombinant proteins recognized native P. falciparum-derived EBA-175, and sera from malaria-immune adults recognized recombinant antigens attesting to both the antigenicity and immunogenicity of proteins. These results suggest that the functionally-active recombinant region II domain of EBA-175 may be an attractive candidate for inclusion in multi-component asexual blood stage vaccines.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 14 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 10
ACCESSION NUMBER: 133:233546 CA
TITLE: Engineering of intracellular sialylation pathways for sialylated glycoprotein production
INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Jarvis, Don; Coleman, Timothy A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA; Johns Hopkins University; University of Wyoming
SOURCE: PCT Int. Appl., 145 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 2000052135 A2 20000908 WO 2000-US5313 20000301
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
AU 2000035083 A5 20000921 AU 2000-35083 20000301
JP 2003524395 T2 20030819 JP 2000-602747 20000301
US 2002142386 A1 20021003 US 2001-930440 20010816
PRIORITY APPLN. INFO.: US 1999-122582P P 19990302
US 1999-169624P P 19991208
WO 2000-US5313 W 20000301
US 2000-227579P P 20000825

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technol. and substrate feeding approaches to enable the prodn. of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examn. of a selection of **insect cell** lines and the use of bioinformatics to identify gene sequences for crit. processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous expression of glycoproteins. Thus, the cDNA for a human **sialic acid** 9-phosphate **synthetase** which produces phosphorylated KDN and **Neu5Ac** from ManNAc-6-P and Man-6-P was cloned and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

L15 ANSWER 15 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 11
ACCESSION NUMBER: 133:291811 CA
TITLE: Cloning and expression of the human N-acetylneuraminic acid phosphate synthase gene with 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid **biosynthetic** ability
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Pitts, Lee R.; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Journal of Biological Chemistry (2000), 275(23), 17869-17877
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Sialic acids** participate in many important biol. recognition events, yet eukaryotic **sialic acid** **biosynthetic** genes are not well characterized. In this study, the authors have identified a novel human gene based on homol. to the Escherichia coli **sialic acid** synthase gene (neuB). The human gene is ubiquitously expressed and encodes a 40-kDa enzyme. The gene partially restores **sialic acid** synthase activity in a neuB-neg. mutant of E. coli and results in N-acetylneuraminic acid (**Neu5Ac**) and 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) prodn. in **insect cells** upon recombinant baculovirus infection. In vitro the human enzyme uses N-acetylmannosamine 6-phosphate and mannose 6-phosphate as substrates to generate phosphorylated forms of

Neu5Ac and KDN, resp., but exhibits much higher activity toward the **Neu5Ac** phosphate product.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 16 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 12

ACCESSION NUMBER: 134:85145 CA

TITLE: Metabolic engineering glycosylation: biotechnology's challenge to the glycobiologist in the next millennium

AUTHOR(S): Warner, Thomas G.

CORPORATE SOURCE: San Carlos, CA, 94070, USA

SOURCE: Carbohydrates in Chemistry and Biology (2000), Volume 4, 1043-1064. Editor(s): Ernst, Beat; Hart, Gerald W.; Sinay, Pierre. Wiley-VCH Verlag GmbH: Weinheim, Germany.
CODEN: 69AMJE

DOCUMENT TYPE: Conference; General Review

LANGUAGE: English

AB A review with 85 refs. The mandate of biotechnol. to develop cost effective, robust recombinant glyco- protein prodn. processes with consistent, complete, and physiol. tolerated glycosylation on therapeutic mols. presents the glycobiologist with near-Herculean challenges for the future. This article summarizes some of the recent developments in glycoprotein carbohydrate metab. and identifies specific genes in these glycosylation pathways that are potential targets for augmentation in order to enhance recombinant protein productivity in currently employed animal cell expression hosts. Similarly, with more prolific prodn. hosts such as **insect cells** or plants, we have detailed the extensive metabolic engineering of the glycosylation pathways that may be necessary to provide the desired glycan structures on the therapeutic. The potential cost benefits offered by these new expression systems is clear justification for pursuing this challenging effort. In this review we have focused on glycosylation in these three expression systems because they are the least complex and most likely to be readily manipulated. Similar metabolic engineering can be considered for yeast and transgenic animals which also have potential as high level expression hosts. In addn., we have defined only those enzyme systems that relate to **biosynthesis** of simple, biantennary N-linked glycans. Similar approaches for metabolic engineering 0-linked glycans as well as more highly branched N- linked structures are also important modifications that should be considered. Although achieving these goals will not be trivial, the resulting effort will undoubtedly be of great benefit, not only for biotechnol., but also for furthering our under- standing of fundamental issues in glycobiol. Recently, over expression of both the sialyltransferase and galactosyl transferase in CHO cells resulted in a near-complete extension of the oligosaccharides on recombinant proteins expressed in this host. This supports the notion that the glycosylation processes are rate limiting in this expression system under high productivity conditions. (Weikert, S. et al., Engineering Chinese hamster ovary cells to maximize **sialic acid** content of recombinant glycoproteins.).

REFERENCE COUNT: 85 THERE ARE 85 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 17 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 13

ACCESSION NUMBER: 132:330031 CA

TITLE: Human follicle stimulating hormone receptor variants lacking transmembrane domains display altered post-translational conformations

AUTHOR(S): Peterson, A. J.; Lindau-Shepard, B.; Brumberg, H. A.; Dias, J. A.

CORPORATE SOURCE: Wadsworth Center, David Axelrod Institute for Public Health, Division of Molecular Medicine, New York State Department of Health, Albany, NY, USA

SOURCE: Molecular and Cellular Endocrinology (2000), 160(1-2), 203-217
CODEN: MCEND6; ISSN: 0303-7207
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Variant splicing of gonadotropin receptor mRNA commonly occurs, however expression of receptor protein variants and their trafficking has yet to be studied in detail. To det. receptor variant trafficking and intracellular processing in mammalian cells, the intracellular fate of intentionally truncated variants of human FSH receptor (hFSH-R) expressed in CHO cells was examd. Monoclonal antibodies (mAbs) were made against the hFSH-R's extracellular domain (ECD) expressed in **insect cells**. Four mAbs 106.156, 106.290, 106.318, and 106.263 were chosen as probes. Epitope mapping using **synthetic** peptides, and truncated hFSH-R variants revealed that mAb 106.156 bound to ECD residues 183-220, while mAbs 106.318, 106.290, 106.263 bound ECD residues 300-331. Immunofluorescence microscopy showed that mAbs 106.318 and 106.156 stained the surface of fixed, intact CHO cells expressing wild type hFSH-R. However, following cell permeabilization all four antibodies stained hFSH-R in Golgi and endoplasmic reticulum. Permeabilized cells expressing truncated variants ECD213 and ECD254 showed staining accumulated in the endoplasmic reticulum/nuclear envelope continuum. ECD335/His was found to accumulate in extended endoplasmic reticulum (ER). The ER location of ECD335/His was confirmed by double labeling expts. with Con A and ECD mAb. Glycosidase digestion followed by Western blot anal. show ECD213 and ECD335/His to be glycosylated, but not ECD254. Both glycosylated truncated hFSH-R variants were sensitive to peptide-N-glycanase F and endoglycosidase H but insensitive to neuraminidase indicating that these variants possess high mannose type oligosaccharides. Thus truncated hFSH-R variants do not reach the medial or trans Golgi where high mannose oligosaccharides are trimmed and **sialic acid** is added.
These data suggest that the conformation the ECD of the wild type receptor is different from the ECD alone expressed in the endoplasmic reticulum. This information suggests that the ECD serves two distinct roles; the first is to bind FSH and the other is likely to contact the endodomain of the receptor, which presumably leads to activation of the endodomain for signal transduction.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 18 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 14
ACCESSION NUMBER: 131:2951 CA
TITLE: O-Glycosylation potential of lepidopteran **insect cell** lines
AUTHOR(S): Lopez, Michel; Tetaert, Daniel; Juliant, Sylvie; Gazon, Maud; Cerutti, Martine; Verbert, Andre; Delannoy, Philippe
CORPORATE SOURCE: UMR CNRS no. 8576, Unite de Glycobiologie Structurale et Fonctionnelle, Laboratoire de Chimie Biologique, Universite des Sciences et Technologies de Lille, Villeneuve d'Ascq, F-59655, Fr.
SOURCE: Biochimica et Biophysica Acta (1998), 1427(1), 49-61
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The enzyme activities involved in O-glycosylation were studied in 3 **insect cell** lines, Spodoptera frugiperda (Sf-9), Mamestra brassicae (Mb) and Trichoplusia ni (Tn) cultured in 2 different serum-free media. The structural features of O-glycoproteins in these **insect cells** were investigated using a panel of lectins and the glycosyltransferase activities involved in O-glycan **biosynthesis** of **insect cells** were measured

(i.e., UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase, UDP-Gal:core-1 .beta.1,3-galactosyltransferase, CMP-NeuAc:Gal.beta.1-3GalNAc .alpha.2,3-sialyltransferase, and UDP-Gal:Gal.beta.1-3GalNAc .alpha.1,4-galactosyltransferase activities). First, we show that O-glycosylation potential depends on cell type. All 3 lepidopteran cell lines express GalNAc.alpha.-O-Ser/Thr antigen, which is recognized by soy bean agglutinin and reflects high UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase activity. Capillary electrophoresis and mass spectrometry studies revealed the presence of .gtoreq.2 different UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases in these **insect cells**. Only some O-linked GalNAc residues are further processed by the addn. of .beta.1,3-linked Gal residues to form T-antigen, as shown by the binding of peanut agglutinin. This reflects relative low levels of UDP-Gal:core-1 .beta.1,3-galactosyltransferase in **insect cells**, as compared to those obsd. in mammalian control cells. In addn., we detected strong binding of Bandeiraea simplicifolia lectin-I isolectin B4 to M. brassicae endogenous glycoproteins, which suggests a high activity of a UDP-Gal:Gal.beta.1-3GalNAc .alpha.1,4-galactosyltransferase. This explains the absence of PNA binding to M. brassicae glycoproteins. Furthermore, our results substantiated that there is no sialyltransferase activity and, therefore, no terminal **sialic acid** prodn. by these cell lines. Finally, we found that the culture medium influences the O-glycosylation potential of each cell line.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 19 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 15

ACCESSION NUMBER: 125:273678 CA

TITLE: Modifying the **insect cell** N-glycosylation pathway with immediate early baculovirus expression vectors

AUTHOR(S): Jarvis, Donald L.; Finn, Eric E.

CORPORATE SOURCE: Dep. Entomol., Texas A&M Univ., College Station, TX, 77843, USA

SOURCE: Nature Biotechnology (1996), 14(10), 1288-1292
CODEN: NABIF9; ISSN: 1087-0156

PUBLISHER: Nature Publishing Co.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The baculovirus-**insect cell** expression system is well-suited for recombinant glycoprotein prodn. because baculovirus vectors can provide high levels of expression and **insect cells** can modify newly **synthesized** proteins in eucaryotic fashion. However, the N-glycosylation pathway of baculovirus-infected **insect cells** differs from the pathway found in higher eukaryotes, as indicated by the fact that glycoproteins produced in the baculovirus system typically lack complex biantennary N-linked oligosaccharide side chains contg. penultimate galactose and terminal **sialic acid** residues. The authors developed a new type of baculovirus vector that can express foreign genes immediately after infection under the control of the viral ie1 promoter. These immediate early baculovirus expression vectors can be used to modify the **insect cell** N-glycosylation pathway and produce a foreign glycoprotein with more extensively processed N-linked oligosaccharides. These vectors can also be used to study the influence of the late steps in N-linked oligosaccharide processing on glycoprotein function. Further development could lead to baculovirus-**insect cell** expression systems that can produce recombinant glycoproteins with complex biantennary N-linked oligosaccharides structurally identical to those produced by higher eukaryotes.

L15 ANSWER 20 OF 20 CA COPYRIGHT 2003 ACS on STN DUPLICATE 16

ACCESSION NUMBER: 113:37898 CA
TITLE: Structure of O-glycosidically linked oligosaccharides **synthesized** by the **insect cell** line Sf9
AUTHOR(S): Thomsen, Darrell R.; Post, Leonard E.; Elhammer, Ake P.
CORPORATE SOURCE: Upjohn Co., Kalamazoo, MI, 49001, USA
SOURCE: Journal of Cellular Biochemistry (1990), 43(1), 67-79
CODEN: JCEBD5; ISSN: 0730-2312
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The O-glycosidically linked oligosaccharides on the pseudorabies virus (PRV) glycoprotein gp50 **synthesized** by 3 different cell lines were studied. The intact membrane protein (gp50) was expressed in Vero cells and in the **insect cell** line Sf9. In addn., a truncated, secreted form lacking the transmembrane and cytoplasmic domains (gp50T), was expressed in CHO and Sf9 cells. The protein, both in intact and truncated form, **synthesized** by the 2 mammalian cells contained only the disaccharide Gal.beta.1-3GalNAc, either unsubstituted or substituted with 1 or 2 **sialic acid** residues. By contrast, the major O-linked structure on gp50 and gp50T **synthesized** by Sf9 cells was the monosaccharide GalNAc. The Sf9 cells also linked lower amts. of Gal.beta.1-3GalNAc to gp50 (12%) and gp50T (26%). None of the structures **synthesized** by Sf9 cells contained **sialic acid**. Measurements of the 2 relevant glycosyltransferases revealed that while all 3 cell lines contain comparable levels of UDP-GalNAc:polypeptide, N-acetylgalactosaminyltransferase activity, there is a greater variation in the levels of UDP-Gal:N-acetylgalactosamine, .beta.1-3 galactosyltransferase, with the Sf9 cells contg. the lowest level.